

Rice Quality by Spectroscopic Analysis: Precision of Three Spectral Regions

F. E. Barton, II,¹ D. S. Himmelsbach,¹ A. M. McClung,² and E. T. Champagne³

ABSTRACT

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Three types of spectroscopy were used to examine rice quality: near infrared (NIR), Raman, and proton nuclear magnetic resonance (¹H NMR). Samples from 96 rice cultivars were tested. Protein, amylose, transparency, alkali spreading values, whiteness, and degree of milling were measured by standard techniques and the values were regressed against NIR and Raman spectra data. The NMR spectra were used for a qualitative or semiquantitative assessment of the amylose/amylopectin ratio by determining the 1-4 to 1-6 ratio for glucans. Protein can be measured by

almost any instrument in any configuration because of the strong relationship between the spectral response and the precision of the reference method. Amylose has an equally strong relationship to the vibrational spectra, but its determination by any reference method is far less precise, resulting in a 10× increase in the standard error of cross-validation (SECv) or standard error of performance (SEP) with *R*² values equal to that of the protein measurement.

The American rice industry is responding to global changes in the export marketplace (Champagne et al 1998). Rice produced in the United States has been perceived to be of lower quality than that produced in Asia and therefore has been priced at a lower value. A study by Kawamura et al (1996) pointed out that “quality”, as perceived by Japanese consumers, is dependent on the location where the rice is grown. High-quality cultivars grown in California were not perceived to be the same quality to the Japanese as cultivars grown in Japan, even though U.S. rice has been and is being successfully marketed in Japan. Differences were ascribed to soil type, fertilization, and “cultural practices”. Quality indices measured included protein, amylose (apparent amylose), translucency, taste, and cooking characteristics. The Kawamura study had one major flaw, it did not address the sample variation (i.e., location effect from one Prefecture to another) of the three rice cultivars grown across Japan and the United States. Champagne et al (1997, 1998) examined the effects of postharvest treatment on the taste and textural properties of rice quality. Degree of milling, which significantly lowered protein content, had the greatest effect on quality. Windham et al (1997) modeled the textural properties of rice with near-infrared spectroscopy (NIRS) and was able to determine textural characteristics equally well with NIR data alone as with sensory, instrumental, reference, and NIR data combined.

Delwiche et al (1996) developed near-infrared models for protein, apparent amylose, milling characteristics, alkali spreading value, and Rapid Visco Analyser (RVA) values. The results for protein were excellent and acceptable for apparent amylose content, milling characteristics, and spreading value. Only the consistency (end-trough) and setback (end-peak) gave reasonable correlations to NIR spectra. These authors tried two spectral geometries and found little difference in the performance of NIRS models. Barton et al (1998a) examined five spectral geometries that included transmission and reflectance measurements and the short wavelength (Herschel region) NIR. The milling characteristics were best modeled in the Herschel region and constituent properties were best modeled in the 1,100–2,500 nm region. Excellent models were developed with limited NIR data, indicating that simple sensors could be developed.

These studies were also conducted on rice sample sets with limited variability. A subsequent study with 96 cultivars was conducted (Barton et al 1997) to measure the milling characteristics, protein, and amylose. Again, good calibrations to protein and apparent amylose were obtained but diversity was still limited, except for three waxy rice samples.

It is apparent that NIRS is not the limiting factor to a standardized protocol for the quality estimation of rice; the reference methods are. As such, research into the true quality of rice is being conducted. This study combined several types of spectral measurement techniques. Near-infrared is the method of choice for rapid cost-effective analyses. Raman spectroscopy offers better structural information that is complementary to NIRS. Solid state proton nuclear magnetic resonance (NMR) spectroscopy can be used to assess amylose/amylopectin branching ratios.

MATERIALS AND METHODS

Samples of 96 individual cultivars of short, medium, and long grain rice were collected from the breeding trials at the Rice Research Unit in Beaumont, TX. All 96 were processed by the Rice Research Unit. Eighty-four samples were grown in California, Texas, Louisiana, or Arkansas; three came from Taiwan, three from Korea; and six from Australia. The 120 samples that were combined with the first 96 samples were as listed earlier (Barton et al 1998a). This gave a robust sample set with a broad range of cultivars, growing conditions, years, milling parameters, drying conditions, and growing locations.

The samples were shelled using a rice machine (model SB, Satake Engineering Co., Tokyo, Japan) and then immediately milled. Regular (light) milling was accomplished using a laboratory mill (Satake one-pass mill pearler, model SKD). The first pass was with a 50-g weight in the 5th position; the second pass was with a 50-g weight in the 3rd position. Milling conditions were 1 min at 1,250 rpm using a fine mesh abrasive wheel. Brokeners were removed with appropriate laboratory-sizing devices using standard indented plates and cylinders. Milling conditions were as reported for the first study (Barton et al 1998a). Samples of ground flour were obtained with a Satake cyclone mill that was heated and equipped with a vibrating trough to admit a steady and uniform supply of rice to the mill.

Reference Analyses of Uncooked Whole Grain Milled Rice

Protein (*N* × 5.95) was determined by the method of combustion in New Orleans, LA, and Athens, GA (AOAC 1990). Apparent amylose content was determined by the method of Juliano (1971) at the Rice Research Unit. Values for whiteness, transparency, and degree of milling were measured (Satake model MM-1B) milling meter in accordance with manufacturer's instructions. Alkali spreading values were determined by the method of Little et al (1958).

¹ USDA-Agricultural Research Service, Richard B. Russell Agricultural Research Center, P.O. Box 5677, Athens, GA 30604. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

² USDA-Agricultural Research Service, Rice Research Unit, Beaumont, TX 77713.

³ USDA-Agricultural Research Service, Southern Regional Research Center, New Orleans, LA 70179.

Additionally, the alkali spreading values were determined by a modified method using a 1.7% KOH solution.

Spectroscopic Analyses

NIR. Three visible/near-infrared scanning monochromators (NIR-Systems model 6500, Silver Spring, MD;) and two Tecator models, a 1265 and a 1229, Silver Spring, MD) were used to collect reflectance and transmittance readings over a wavelength range of 400–2,498 nm or 850–1,050 nm. The instruments were operated by either the software package NIR3 v.3.11 (Infrasoft International, Inc., Port Matilda, PA), which includes modules for acquisition and processing of spectra or Tecator's data collection modules and transferred to ISI format for processing. Whole grain milled rice (100 g) was scanned in reflectance mode on a NIRSystems 6500 instrument in a transport cell as described by Delwiche et al (1996) and Barton et al (1997). Whole grain milled rice (150 g) was scanned in transmittance mode in the standard sample cup for the Tecator 1265, and 250–300 g whole grain milled rice samples were passed through the whole grain cell of the Tecator 1229. Additionally, whole grain milled rice and ground flour (3.0 g) were scanned in the NIRSystems 6500 in a 2-in. sample holder in the spinning cup module.

Raman. Raman spectra were obtained from two subsamples of whole grain milled rice and ground flour using an FT-Raman spectrometer (Nicolet Analytical Instruments 950) interfaced to a PC running Nicolet OMNIC (v 4.1) software. The same samples

used for the NIR spinning cup spectra were used in a locally fabricated spinning device for the Raman spectrometer. Thus the same samples were scanned by both instruments. A laser light source of 1.064 μm at 500 mW of power was used with a CaF_2 beamsplitter and a Ge detector. The data obtained were the result of 128 scans at a resolution of 16 cm^{-1} . Interferograms were processed into the frequency domain with Happ-Genzel apodization. The spectra of subsamples were averaged to give a single spectral file for each sample. No attempt was made to remove fluorescence from the spectra, and only the Stokes region of the spectrum (4,000–200 cm^{-1}) was utilized.

NMR Analysis. Rice samples were packed into a 4 mm diameter CPMAS rotor with Pyrex spacers to contain the sample to an approximately spherical volume in the center of the rotor, the effect of which is to increase magnetic field homogeneity of the sample volume, thus increasing spectral resolution. The rotors were sealed with a Teflon gasket to prevent evaporative loss and spun at 2 kHz in a CPMAS probe. Solid state ^1H NMR experiments were performed using a Spectrospin 7.05 T magnet with a proton frequency of 300.13 MHz. A simple one-pulse, phase-cycled pulse sequence was used to acquire the ^1H NMR data, with a sweep width of 4,000 Hz and a 4 sec recycle delay.

Multivariate Analyses

Commercial spectral analysis programs (Unscrambler, v 5.5, CAMO, Trondheim, Norway; GRAMS ver 5.0 form Galactic, Inc. Salem, NH; Turbo-Quant, Nicolet Instruments, Madison, WI, and NIR3 ver 4.0, InfraSoft International, Inc. Port Matilda, PA) were used to process the data from constituent analyses and to develop chemometric models. For the NIR, the preprocessing data technique of multiplicative scatter correction (MSC) (Isaksson and

TABLE I
Summary of Chemical and Satake Milling Meter Constituents in Milled Rice^{a,b}

Constituent	Range	Mean	Standard Deviation
Amylose (%)	16.40–23.50	19.42	1.51
Protein (%)	3.90–8.30	5.98	0.88
Taste value	50.00–85.00	73.93	11.09
Whiteness	32.30–52.00	43.93	5.59
Transparency	2.41–4.69	3.28	0.43
Degree of milling	60.00–157.00	116.72	26.24

^a % dry matter basis.

^b From Barton et al (1998a).

TABLE II
Standard Error of Cross Validation for Whole-Grain Milled Rice and Rice Flour for Different Sample Geometries and Software Systems^{a,b}

Constituent	6500 Transport	6500 Spinning Cup	6500 Spinning Cup Flour	6500 RTCAL	1265
Amylose	0.52	0.55	0.53	0.59	0.53
Protein	0.22	0.25	0.18	0.22	0.18
Taste value	3.22	3.85	3.88	4.15	4.32
Whiteness	0.65	0.99	1.51	0.59	1.01
Transparency	0.13	0.15	0.17	0.17	0.19
Degree of milling	2.89	3.92	5.71	2.55	4.29

^a From Barton et al (1998a).

^b NIRSystem model 6500 with transport sample and spinning cup configuration; Tecator model 1265 transmission instrument.

TABLE IV
Standard Error of Cross-Validation for Whole-Grain Milled Rice and Rice Flour for Combined Files from 1996 and 1997

Constituent	6500 Transport	6500 Flour Spinning Cup	1229 SECv	1229 SEP
Amylose	1.17	0.53	1.83	1.90
Protein	0.22	0.14	0.20	0.22
Whiteness	0.71	1.76	1.54	1.72
Transparency	0.20	0.22	0.24	0.29
Degree of milling	3.13	7.69	6.01	7.46

TABLE V
Standard Error of Cross-Validation for Rice Flour by Raman Spectroscopy

Constituent	SECv	R ²
Amylose	1.40	0.99
Protein	0.15	0.98
Whiteness	2.18	0.74
Transparency	0.46	0.67
Degree of milling	7.98	0.89
1.5% alkali	0.97	0.87
1.7% alkali	0.71	0.79

TABLE III
Standard Error of Cross-Validation for Diverse Rices in Four Optical Geometries for Eight Constituents^a

Constituent	Mean	Minimum	Maximum	1229	1265	6500 Transport	6500 Spinning Cup
Amylose	18.44	0.41	24.9	1.76	1.79	2.64	1.25
1.5% alkali	4.51	2.00	7.0	0.81	0.75	0.99	0.99
1.7% alkali	5.27	2.10	7.0	0.84	0.73	0.64	0.67
Whiteness	40.3	33.30	47.5	1.56	1.46	0.70	1.92
Transparency	2.74	0.52	3.79	0.26	0.28	0.19	0.35
Degree of milling	96.6	64	143	6.75	5.92	3.34	8.10
Protein	8.07	7.03	10.0	0.16	0.16	0.19	0.14
Protein + amylose	26.61	7.45	32.78	1.79	1.79	2.73	1.23

^a NIRSystem model 6500 with transport sample and spinning cup configuration; Tecator models 1229 and 1265 transmission instruments.

Naes 1988) was applied to the spectra to remove interferences from scatter and then transformed with a second derivative (gaps of 1–12 nm were tried; a gap of 4 nm was chosen and subsequently used for all comparisons) to enhance absorption bands. The multivariate methods of partial least squares (PLS1) as described by Martens and Naes (1989) were used for predicting dependent variables from NIRS spectra, chemical/Satake milling meter values, and instrumental spectral intensities. The concepts and properties of PLS in relation to NIRS were discussed by Martens and Naes (1989). The calibration models (PLS1) were validated using full cross-validation where each sample was used to test the model estimated by all other samples. For Raman spectra, the data were preprocessed by using the mean centering techniques from Unscrambler. Because Raman is a scattering technique, any form of scatter correction (MSC or derivative) was not effective in improving the models, in fact it was detrimental in most instances. The predicted error sum of squares (PRESS) was the used to determine the number of PLS1 factors for the model. In all cases, the number of factors selected was between 6 and 8, except Raman protein, which required only 4 factors and milling degree in NIR and Raman for which only 3 factors were selected.

RESULTS AND DISCUSSION

In a preliminary report, the results of an investigation of the use of Raman spectroscopy to measure the constituent parameters and quality indices of rice were given by Barton et al (1997). At this time, the concept of using SS ^1H NMR spectroscopy to measure the 1–4/1–6 ratio of amylose and amylopectin was also introduced as a possible way to better describe rheological properties. The data sets have been expanded for this report to include two years; the results are shown in Tables I–IV. The data from the first year is in Table I and II, and the treatments include four cultivars at different milling and drying parameters. The results (the standard error of

cross-validation [SECv]) of the models generated from this data are extremely good, but the file is not very diverse with milling degree being the parameter that separates the quality (Barton et al 1998a). Windham et al (1997) reported similar results when examining the textural properties of these rice samples. Table III contains the results of the second year study with very diverse rices. The range of compositional and milling properties is broader than the first year and the results, particularly for amylose, are less precise. Table IV contains the data from the combination of the two years data. For the first time, a single geometry is clearly better: the NIRSystems 6500 with the transport sample compartment and in reflectance mode. This indicates that still more spectral data is required to model the more diverse population.

The Raman data in Table V, using only the second year's data shows the method to be comparable to NIRS in most instances. Calibration for protein is quite good and is an improvement over the data we reported last year in preliminary results (0.15 vs. 0.24 SECv). Figure 1 shows the Raman spectra of three rice samples: one waxy (0.5–3% amylose), one low amylose (11–12% amylose), and one high amylose (>20% amylose). The waxy rice has the higher absorbances in the C–H stretch ($2,900\text{ cm}^{-1}$) region and lower amounts of C–O stretch at 941 cm^{-1} . The Raman spectra is "more spectrally rich" than the NIR spectra of rice, and could give equivalent or better results to NIRS with very small Raman spectral windows as shown by Archibald et al (1998) for flax fiber. The Raman is very insensitive to water, so any correlation to alkali spreading value was a surprise. The alkali spreading value is a measure of gelatinization, thus water absorption. The NIRS work of Delwiche et al (1996) describes the correlation to the 1.5% KOH values with an SEP of one-half the SECv of the Raman data in Table V (0.48 vs. 0.97) and R^2 about the same (0.82 vs. 0.87). Wadsworth (1993) has shown the correlation between alkali spreading

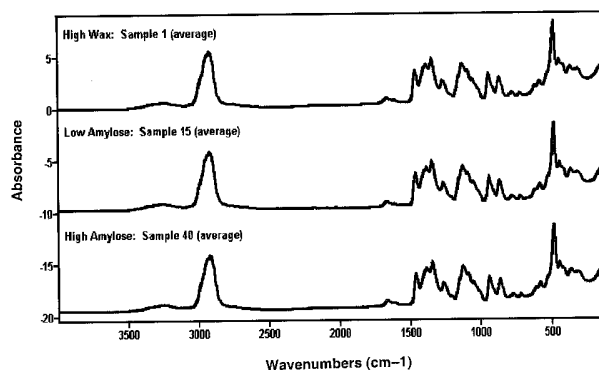


Fig. 1. Fourier transform-Raman spectra of waxy low- and high-amylose rice samples.

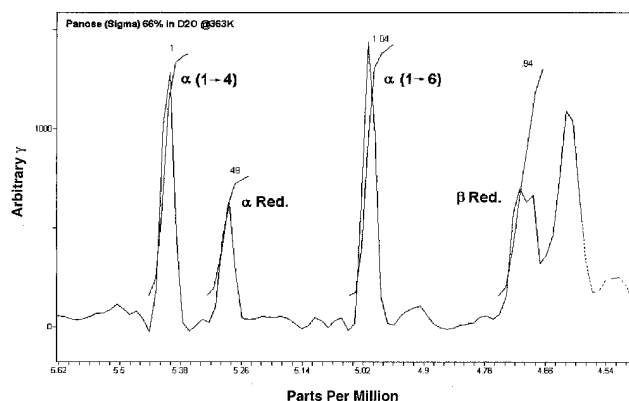


Fig. 2. ^1H nuclear magnetic resonance (NMR) spectrum of Panose.

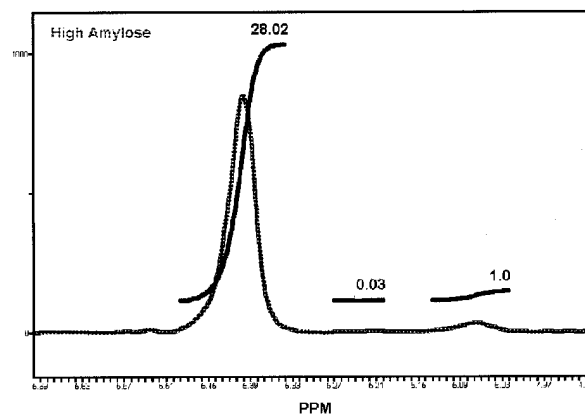


Fig. 3. ^1H MAS nuclear magnetic resonance (NMR) of high-amylose rice flour.

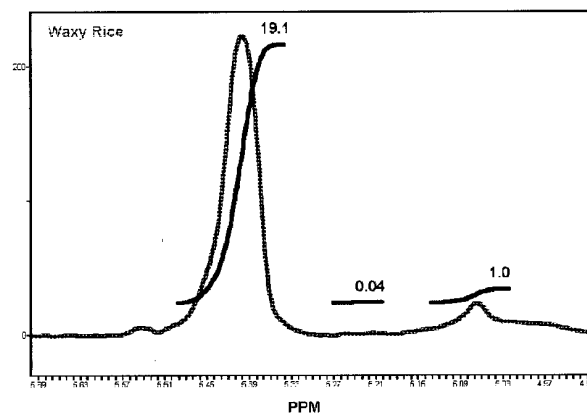


Fig. 4. ^1H HR MAS of waxy rice flour.

values and the NIR spectrum of rice to be mainly to water bands at 1,930 and 1,445 nm. The results of the 1.7% KOH correlation ($SECv = 0.71$ and $R^2 = 0.79$) indicates that the stronger alkali may bring the values for extreme samples closer to the regression line, but overall the data contains more scatter. These results will also be part of further study.

The measurement of the starch fraction of rice is a difficult assay. The amylose-amylopectin matrix is very tightly bound with proteins, more so than in wheat or corn. In our laboratory, extractions to remove amylose from rice samples usually leave a significant portion of starch glued to the sides of a flask by protein. Efforts to digest protein away from the starch usually result in starch being lost as well. Any assay which then determines amylose is compromised by this loss. One has to assume that the amylose and amylopectin are lost in their respective proportions. But this is not a good assumption and one reason why the assay does not yield results that agree as well as one would expect from the NIR correlations. A spectroscopic assay that determines starch by its molecular bonds would be most useful. The principal vibrations for amylose are the number one carbon in the $\alpha 1-4$ linked carbohydrate and normal C-H stretch. The principal vibrations for amylopectin are the number one carbon in the $\alpha 1-6$ linked carbohydrate, $\alpha 1-4$, and C-H. The cleanest way to observe the spectral response of these carbons is from their solid state proton NMR spectra (Fig. 2). The $\alpha 1-4$ is easily distinguished from the 1-6 in panose which is an ideal model compound because the 1-4/1-6 ratio is 1:1. A method of correlating this ratio to starch composition, RVA values, alkali spreading values, and the NIR and Raman spectroscopy is being conducted. The spectra of a high amylose (Fig. 3) and a waxy rice (Fig. 4) show the 1-4/1-6 ratios calculated as 28.02 for high amylose and 19.2 for the waxy sample. It is also possible to obtain a very rough approximation of chain length from the integrated area of the alpha reducing end. In this case, the high amylose chain length is $\approx 25\%$ longer than that of the waxy rice. A typical chain length for amylopectin is $\approx 25-30$ units.

CONCLUSIONS

It is apparent that protein can be measured by almost any instrument in any configuration because of the strong relationship between the spectral response and the precision of the reference method. Amylose has an equally strong relationship to the vibrational spectra, but its determination by any reference method is far less precise, thus we see an increase in the $SECv$ or SEP of almost 10% with R^2 equal to that of the protein measurement. The relationship between spectra and the 1.5 % alkali values are better than the 1.7% alkali, but the lower R^2 of the 1.7% alkali suggests it is a less precise extractor. This is the same for both NIRS and Raman. The measurement of 1-4 to 1-6 ratio for amylose amylopectin by NMR could yield a better measure of starch quality for end use.

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